Keten and Buehler Reply: We recently proposed a fracture mechanics model to predict an upper limit for the rupture strength of a polypeptide chain stabilized by H bonds at vanishing pulling rates [1]. In the preceding Comment [2], Makarov raises the concern that in the shear loading scenario discussed in [1], the force prediction should actually be infinite. Here, we emphasize that our original derivation is correct and point out the differences between our approach and that presented in [2]. According to Eq. (1) in [2], the free energy of the system can be written as $A(x, \lambda) = \lambda A_{\mathrm{WLC}}(x/\lambda) - F(x - \lambda) - \gamma_s(L - \lambda)$. This requires that the attached polypeptide chain is fully extended, which differs from our assumptions [1]. Our assumption is that the bonded chain is fully relaxed, in which case λ in the force term in Eq. (1) in [2] disappears, leading to $A(x, \lambda) = \lambda A_{\mathrm{WLC}}(x/\lambda) - Fx - \gamma_s(L - \lambda)$. This assumption is evident both from the fact that free energies are consistently calculated with respect to the fully relaxed state [$A_{\mathrm{WLC}} = \int_0^{\alpha} F_{\mathrm{WLC}}(\alpha)d\alpha$] and from the expression for the work done by the external force. In [1], the variable dx corresponds to the displacement of the chain end, and the integration is performed with respect to a point that remains stationary. While our assumption may be considered limiting, it is not methodologically incorrect as argued in [2]. On the contrary, our original model [1] represents a minimal theory that has the least number of parameters and yields the simplest analytical formulation possible.

The underlying assumptions between Makarov's approach and our derivation represent two extreme cases. In general, the attached segment of the chain would have a finite average end-to-end distance given as sL, where $0 < s \leq 1$ and L is the contour length of the attached segment (see inset, Fig. 1). Then Eq. (1) in [2] becomes $A(x, \lambda) = \lambda A_{\mathrm{WLC}}(x/\lambda) - F(x - s\lambda) - \gamma_s(L - \lambda) + (L - \lambda)A_{\mathrm{FIX}}(s)$ where the last term $A_{\mathrm{FIX}} = \int_0^s F_{\mathrm{WLC}}(\alpha)d\alpha$ has to be introduced since free energy integrals are taken with respect to the zero-stretch state, whereas the liberated segment already has a prestretch (noting that we treat entropic elasticity and H bond terms separately). If this consideration were included in Makarov's expression, it would yield a rupture strength value of zero as the WLC model diverges at full extension. Quantifying the strength with assumptions on initial extension state of the bonded chain is thus clearly not straightforward.

Proceeding as we did previously [1] but with the prestretch accounted for, we substitute $\alpha = x/\lambda$ and solve for the critical α that satisfies $A_{\mathrm{WLC}}(\alpha_{\mathrm{cr}}) + F(s - \alpha_{\mathrm{cr}}) + \gamma_s - A_{\mathrm{FIX}}(s) = 0$, $F_{\mathrm{WLC}}(\alpha_{\mathrm{cr}}) = F_{\mathrm{cr}}$. For $E_b = 4$ kcal/mol, $\xi_p = 0.4$ nm, variations of s in the range $0 \leq s \leq 0.6$ yield results within the error range of results shown in Fig. 4 in [1]. The case $s \rightarrow 1$ (specific to beta-sheet systems) would involve the possibility of stick-slip motion as a key failure mechanism (H bonds reform after sliding). We evaluate the critical condition as $2A_{\mathrm{WLC}}(\alpha_{\mathrm{cr}}) + F(s - 2\alpha_{\mathrm{cr}}) + \gamma_s - 2A_{\mathrm{FIX}}(s) = 0$ and extrapolate results to the case $s \rightarrow 1$ (Fig. 1). These results are in excellent agreement with our original prediction, illustrating the robustness of the results reported in [1]. Specifically, our results shown in Fig. 1 here and Fig. 4 in [1] (both lead to $\approx$127 pN) agree well with experiments (Fig. 1 in [1]). Typical rupture forces (the case considered here, distinct from refolding) in force-quenching experiments [3] agree with our predictions. The number of H bonds present and the loading geometry will affect the strength of the assembly as discussed in [1]. The predictions reported in [1] correspond to an upper strength limit of a large uniformly loaded cluster of H bonds.

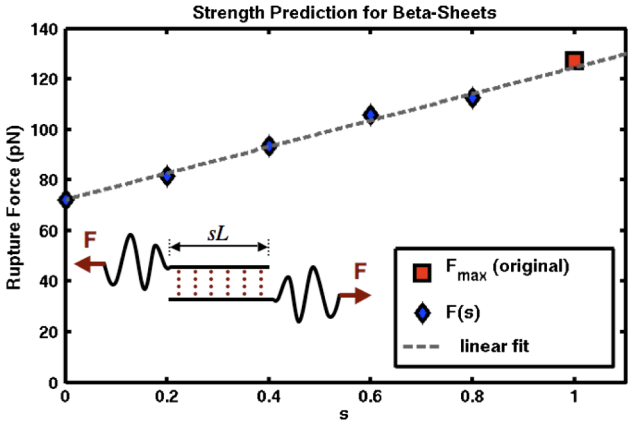

FIG. 1 (color online). Strength prediction for varying values of s, including an extrapolation to $s = 1$ (double stranded slip case). The linear extrapolation is done for $0 \leq s \leq 0.8$ (values admissible to the WLC model). The value for $s \rightarrow 1$ approaches the prediction by our original model [1], illustrating the robustness of the main contribution of our Letter with respect to different physical boundary conditions. The inset shows the double strand shear condition and parameter s, defined as the ratio of end-to-end length to contour length for the attached segment.

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